REMARKS

Claims 1-62 are pending. Claims 1-15 and 43-62 are under examination. Claims 1, 16 and 58 have been amended, and new claims 63 and 64 have been added. Support for the amendment and new claims can be found throughout the specification and the claims as filed. Claim 1 has been amended to merely incorporate language from the preamble into the body of the claim. Claims 16 and 58 have been amended to independent form. Support for new claims 63 and 64 can be found, for example, in original claim 6. Accordingly, these amendments and new claims do not raise an issue of new matter and entry thereof is respectfully requested.

Regarding the Power of Attorney, it is respectfully pointed out that a Revocation and Power of Attorney was submitted with the response mailed December 29, 2003, appointing attorneys and agents at McDermott Will & Emery LLP, 4370 La Jolla Village Drive, Suite 700, San Diego, California 92122. It is respectfully requested that the Power of Attorney be acknowledged and that future correspondence be forwarded to the indicated address.

Rejections Under 35 U.S.C. § 112, First Paragraph

The rejection of claims 1-15 and 43-57 under 35 U.S.C. § 112, first paragraph, as allegedly lacking sufficient written description is respectfully traversed. Applicants respectfully submit that the specification provides sufficient description and guidance to convey to one skilled in the art that Applicants were in possession of the claimed invention at the time the application was filed.

The Office Action indicates that there are many species of sesquiterpene lactones, diterpene lactones and triterpene lactones, referring to Harborne et al., Phytochemical Dictionary 2nd ed., pp. 654-657, Taylor & Francis Inc., Philadelphia (1999). The Office Action asserts that Applicants have not provided evidence that they were in possession of every sesquiterpene lactone listed in the Phytochemical Dictionary and asserts that this was evidence that Applicants were not in possession of every combination of sesquiterpene lactone and diterpene lactone or every combination of sesquiterpene lactone and triterpene.

Applicants respectfully submit that the specification provides sufficient description and guidance for the claimed compositions. The specification teaches generic formulae for sesquiterpene lactones, diterpene lactones and triterpenes (see Figures 1 and 2) as well as numerous exemplary species (see, for example, Table 1 on page 13).

With regard to the assertion that Applicants have not provided evidence of possession of every sesquiterpene lactone listed in the Phytochemical Dictionary, the Examiner is reminded that the Federal Circuit has held that "a specification need not disclose what is well known in the art" *Genentech Inc. v Novo Nordisk A/S*, 108 F.3d 1361, 1366, 42 U.S.P.Q.2d 1001, 1005 (CAFC 1997) quoting *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1385, 231 U.S.P.Q. 81, 94 (Fed. Cir. 1986). Indeed, the written description requirement for a claimed genus can be satisfied through sufficient description of a representative number of species. Moreover, the MPEP indicates that "[D]escription of a representative number of species does not require the description to be of such specificity that it would provide individual support for each species that the genus embraces" (MPEP § 2163 II (3)(a)(ii)).

An objective standard for determining compliance with the written description requirement is, "does the description clearly allow persons of ordinary skill in the art to recognize that he or she invented what is claimed." *In re Gosteli*, 872 F.2d 1008, 1012, 10 USPQ2d 1614, 1618 (Fed. Cir. 1989). Applicants respectfully submit that, based on the teachings in the specification and what was well known in the art, one skilled in the art would have readily recognized that Applicants invented the claimed compositions.

Applicants submit that the specification provides sufficient description and guidance for the claimed compositions. Accordingly, Applicants respectfully request that this rejection be withdrawn.

The rejection of claims 1-15 and 43-57 under 35 U.S.C. § 112, first paragraph, as allegedly lacking enablement is respectfully traversed. Applicants respectfully submit that the specification provides sufficient description and guidance to enable the claimed compositions.

In the Office Action on pages 8-9, the following is asserted:

> Inventions targeted for human therapy bear a heavy responsibility to provide supporting evidence because of the unpredictability in biological responses to therapeutic treatments. The standard of enablement is higher for such inventions because effective treatments for disease conditions are relatively rare, and may be unbelievable in the absence of strong supporting evidence. Claims drawn to pharmaceutically acceptable compositions and to methods of administering compounds to humans generally require supporting evidence because of the unpredictability in biological responses to therapeutic treatments.

Applicants respectfully disagree with the assertions in the Office Action set forth in the above-recited paragraph. Applicants' representative is not aware of the precedent for such an assertion with respect to human therapy and would appreciate being provided with the relevant authority so that the case law can be reviewed and responded to appropriately. To the contrary, Applicants' representative is aware of the legal precedent for enablement based on *in vitro* and *in vivo* models having reasonable correlation with *in vivo* applications (see *In re Brana*, 51 F.3d 1560, 1566, 34 USPQ2d 1436, 1441 (Fed.Cir. 1995); *Cross v. Iizuka*, 753 F.2d 1040, 1050, 224 USPQ 739,747 (Fed. Cir. 1985). Applicants respectfully submit that the models taught in the specification (see Examples on pages 23-50) are sufficient to enable *in vivo* applications of the claimed compositions. Moreover, Applicants respectfully submit that the billions of dollars earned by the pharmaceutical industry belies the assertion in the Office Action that effective treatments for disease conditions are relatively rare and are therefore unbelievable.

Applicants respectfully submit that the specification provides sufficient description and guidance to enable one skilled in the art to make and use the claimed compositions without undue experimentation. In the Office Action, it is asserted that there are hundreds of known sesquiterpene lactones having respective structures and functions and that these are unpredictable. Applicants point out that the claimed compositions contain sesquiterpene lactones that share structural and functional characteristics in that they have the structure of sesquiterpene lactones and function to inhibit inducible COX-2 activity and have minimal effect on COX-1 activity.

Applicants respectfully disagree with the assertion in the Office Action on page 9 that "the prior art does not provide the artisan any reasonable expectation that any one material embodiment of the claimed invention would be more likely than not to function in the manner disclosed." Applicants respectfully maintain that, based on the teachings in the specification and what was well known in the art, one skilled in the art would have expected the claimed compositions, which are directed to compositions having specifically recited functional characteristics of inhibiting COX-2 activity and having minimal effect on COX-1 activity, to function as claimed. The specification teaches compositions containing a sesquiterpene lactone species and either a diterpene lactone species or triterpene species, where the composition inhibits inducible COX-2 activity and has minimal effect on COX-1 activity, as well as exemplary species (page 9, lines 6-16; page 11, lines 11-21; page 12, lines 10-15; and Table 1 on page 13). The specification further teaches methods of measuring the claimed functional activity (see Examples, page 23-50). Therefore, in contrast to the assertion in the Office Action, Applicants contend that one skilled in the art would have had a reasonable expectation that the compositions would function as claimed. In corroboration of Applicants' assertion, attached herewith is a reference by Hwang et al., Biochem. Biophys. Res. Comm. 226:810-818 (1996)(Exhibit A). Hwang et al. exemplifies that various sesquiterpene lactones inhibit COX-2 activity (see Table 1, page 816), and it is respectfully submitted that Hwang et al. corroborates Applicants' position. Therefore, contrary to the assertion in the Office Action, Applicants respectfully maintain that one skilled in the art would have had a reasonable expectation that the claimed embodiments would function as recited in the claims.

Applicants maintain that the specification provides sufficient description and guidance to enable the claimed compositions. Accordingly, Applicants respectfully request that this rejection be withdrawn.

Allowable Subject Matter

The Office Action indicates that claims 16-20 and 58-62 are objected to as depending from a rejected claim but would be allowable if rewritten in independent form.

Applicants point out that claims 16 and 58 have been rewritten in independent form. Accordingly, claims 16-20 and 58-62 should be allowable.

CONCLUSION

In light of the amendments and remarks herein, Applicants submit that the claims are now in condition for allowance and respectfully request a notice to this effect. The Examiner is invited to call the undersigned agent if there are any questions.

To the extent necessary, a petition for an extension of time under 37 C.F.R. 1.136 is hereby made. Please charge any shortage in fees due in connection with the filing of this paper, including extension of time fees, to Deposit Account 502624 and please credit any excess fees to such deposit account.

Respectfully submitted,

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SDO 14273-1.068911.0018

BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS 226, 810–818 (1996)
ARTICLE NO. 1433

Inhibition of the Expression of Inducible Cyclooxygenase and Proinflammatory Cytokines by Sesquiterpene Lactones in Macrophages Correlates with the Inhibition of MAP Kinases

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In our previous studies (Refs. 1 and 2), it was shown that protein tyrosine kinase (PTK) inhibitors, radicicol and herbimycin A, inhibit the expression of the mitogen-inducible cyclooxygenase (COX-2) and proinflammatory cytokines. Radicicol and herbimycin A possess polarized double bonds which can conjugate sulphydryl groups of proteins. Parthenolide, the predominant sesquiterpene lactone in European feverfew (*Tanacetum parthenium*), contains α-methylene-gamma-lactone (MGL) and an epoxide in its structure. These moieties can interact with biological nucleophiles such as a sulfhydryl group. Parthenolide inhibited the expression of COX-2 and proinflammatory cytokines (TNFα and IL-1) in lipopolysaccharide (LPS)-stimulated macrophages. The structure-function relationship indicates that the MGL moiety confers the inhibitory effect. Parthenolide suppressed LPS-stimulated protein tyrosine phosphorylation in the murine macrophage cell line (RAW 264.7). This suppression was correlated with its inhibitory effect on the expression of COX-2 and the cytokines. Among tyrosine phosphorylated proteins, mitogen-activated protein kinases (MAPKs) exhibited the most dramatic inhibition.

Protein-tyrosine kinases (PTK) play a key role in the regulation of cell proliferation and differentiation (3,4). Many growth factor receptors and oncogene products possess PTK activity. These receptor type and non-receptor type PTK participate in transmembrane and intracellular signal transduction pathways, respectively. Abolishing PTK activity of some of these receptors by site-directed mutagenesis resulted in elimination of their biological activity (5,6). It has been demonstrated that PTK inhibitors suppress growth and revert the morphology of cells transformed by oncogenes encoding PTKs such as src, yes, fps, erB2 (7). For this reason, enhanced interest has been generated to investigate PTK inhibitors as a potential therapeutic agent for cancers (8). In addition, lipopolysaccharide (LPS) stimulated protein tyrosine phosphorylation in macrophages (9) and induced the expression of the mitogen-inducible cyclooxygenase (COX-2) and TNFα (10,11). PTK inhibitors suppressed the expression of cyclooxygenase and TNFα in macrophages (1,2,10). The synthetic PTK inhibitors, tyrphostins, were shown to prevent LPS-induced lethal toxicity in mice (10). These results suggest the possibility that PTK inhibitors may be an effective therapeutic agent for septic shock and other acute inflammatory diseases.

The PTK inhibitors derived from natural products include quercetin (12), genistein (13) levendustin A (14), erbstatin (15) and herbimycin A (16). Synthetic PTK inhibitors include tyrphostins which contain the benzylidene moiety of erbstatin and other arylidene compounds (8), and a specific inhibitor of the epidermal growth factor receptor tyrosine kinase (17). We report here that parthenolide, inhibits the expressions of proinflammatory cytokines and COX-2. This inhibition was correlated with its suppressive effect on protein tyrosine phosphorylation, particularly those of MAPKs, in macrophage.

0006-291X/96 $18.00

EXHIBIT A

MATERIALS AND METHODS

Preparation of parthenolide and other sesquiterpene lactones. Parthenolide (compound 1 in Fig. 1) was extracted from dried leaves of *Magnolia grandiflora* as described originally by El-Feraly and Chan (18). Structural identity of parthenolide and other sesquiterpene lactones were determined spectroscopically (^1H and ^{13}C NMR, IR, MS) as described previously (19).

Isolation of macrophage. Rat (Sprague-Dawley) alveolar macrophages were collected by broncho-alveolar lavage as described previously (11). The murine macrophage cell line, RAW 264.7 (ATCC), was cultured in Dubecco's modified Eagle's medium (DMEM) containing 10% heat-inactivated fetal bovine serum (FBS, Intergen). For the cyclooxygenase activity assay, cells were seeded in 24 well plates, and after near confluency cells were treated with aspirin (250μM) for 2.5h to inactivate endogenous cyclooxygenase. The time course for the COX activity indicated that the maximum increase was reached in 8h (Data not shown). COX activity was determined by measuring prostaglandin E_2 concentrations in cells incubated with arachidonic acid (30μM) for 10 minutes as described previously (11).

Antiphosphotyrosine immunoblotting. This was carried out essentially the same as previously described (1) using 4G10 monoclonal antiphosphotyrosine antibody (UBI) and the ECL detection system (Amersham).

Western blot analyses for COX-2, interleukin-1α (IL-1α), c-Jun N-terminal kinase-1 (JNK-1) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) proteins. The protein levels of COX-2 and GAPDH were assessed by Western blot analysis using polyclonal antibodies as described previously (11). Polyclonal antibodies for IL-1α and JNK-1 were purchased from Genzyme and Santa Cruz Biotech, respectively.

RNase protection assay. Total cellular RNA was isolated by TRIzoL reagent (Gibco, BRL). The RNase protection assay was performed as described previously (1).

In-gel kinase assay. This was performed according to the method described by Kameshita et al. (20) using myelin basic protein (MBP) as a substrate.

RESULTS AND DISCUSSION

Inhibition of the expression of cyclooxygenase and proinflammatory cytokines by parthenolide in LPS-stimulated alveolar macrophage. In our previous study, it was shown that increased COX activity in LPS-stimulated macrophage results from selective expression of COX-2 (11). Therefore, recovered COX activity in cells pretreated with aspirin reflects *de nevo* synthesized COX-2. The dose-response to parthenolide in inhibiting the expression of COX activity showed that the IC_{50} is 0.8μM as shown in Fig. 2. Similar inhibitions of the expression of COX-2 protein and steady state levels of COX-2 mRNA are shown in Fig. 3. Whether the suppression of the steady state levels of COX-2 mRNA by parthenolide is due to the inhibition of transcription rate or accelerated degradation of mRNA is not known. In our previous studies,it was shown that radicicol, protein tyrosine kinase inhibitor, accelerates the degradation of mRNA for COX-2, TNFα and IL-1β (2). Parthenolide suppressed LPS-induced TNFα production with an IC_{50} of 0.1μg/ml (Fig. 2). Steady state levels of mRNA for TNFα and IL-1β were also inhibited by parthenolide (Fig. 4). Parthenolide inhibited the expression of IL-1α protein (non-secreted precursor form of IL-1α) as determined by western blot analysis (Fig. 3).

Proinflammatory cytokines and COX-2 belong to immediate early gene family. In our previous studies, it was shown that PTK inhibitors suppress the expression of many immediate early genes except protein tyrosine phosphatase (PTPase, 3CH134) in LPS-stimulated rat alveolar macrophage (2). Significance of the resistance of PTPase to PTK inhibitors is not known. Parthenolide also did not inhibit the expression of PTPase (Fig. 4). 3CH134 is a growth factor-inducible immediate early gene that encodes a dual specificity MAPK phosphatase, MKP-1 or 3CH134 (21). This phosphatase inactivates MAPKs by dephosphorylating both their phosphorthreonine and phosphotyrosine residues (22). The inactivation of MAPKs by 3CH134 is implicated in providing a feedback loop to terminate growth factor-induced signals.

Parthenolide suppresses tyrosine phosphorylation of proteins including the mitogen-activated protein kinases (MAPKs) in RAW 264.7 cell. The stimulation of macrophages

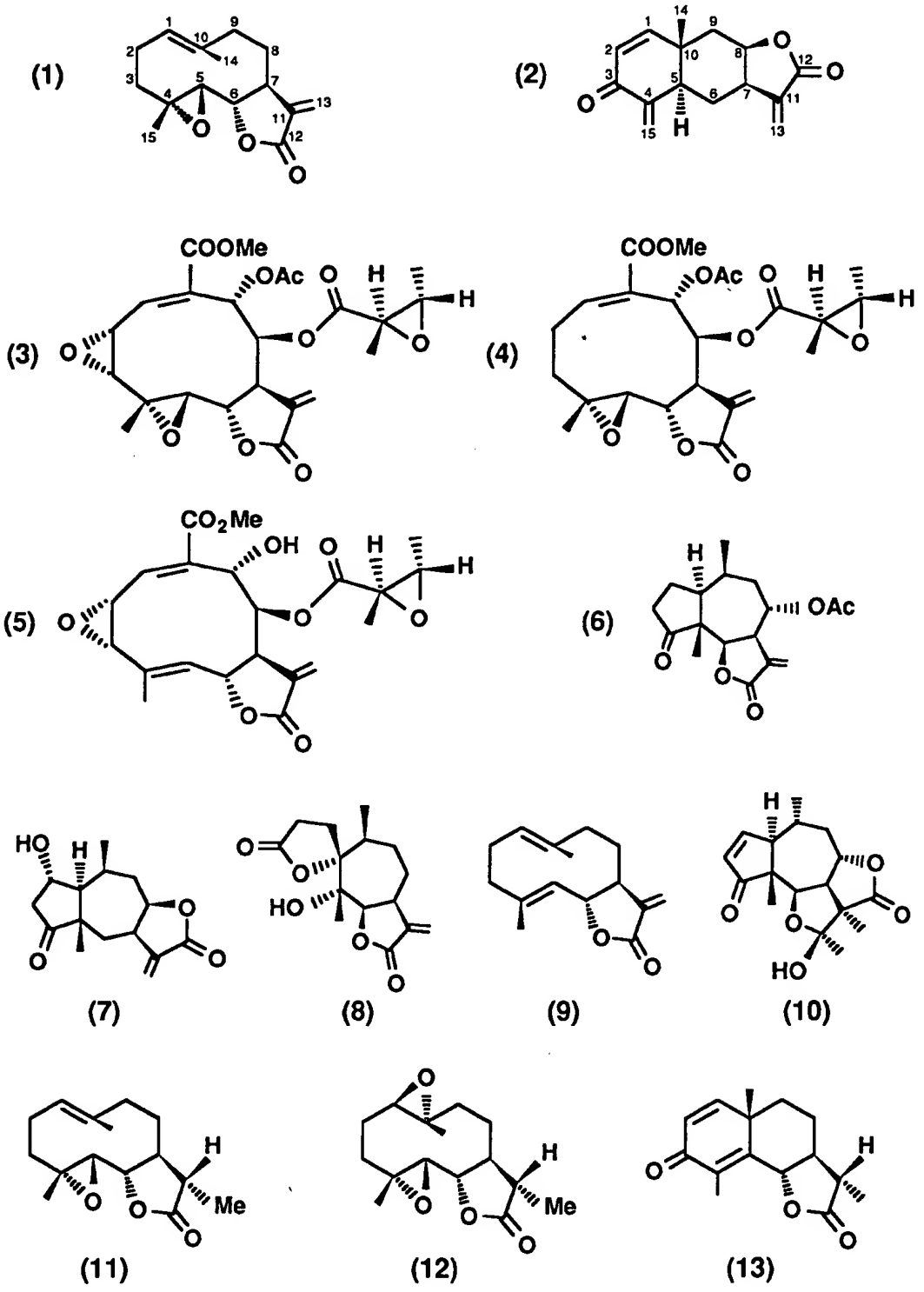

FIG. 1. Chemical structure of parthenolide and other sesquiterpene lactones.

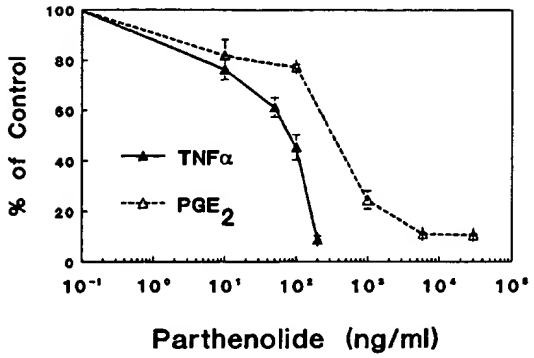

FIG. 2. The dose-response by parthenolide in inhibiting *de novo* synthesis of COX or TNFα in LPS-stimulated macrophages. Alveolar macrophages pretreated with aspirin were incubated with LPS (10 μg/ml) and various concentrations of parthenolide for 16h. The activity of *de novo* synthesized COX was determined by measuring the levels of PGE_2 produced from exogenous arachidonic acid. Activity of TNFα was determined by bioassay using L929 cell (26). Values are the means of triplicate samples for TNFα and duplicate samples for PGE_2.

by LPS results in activation of members of MAPKs (9) that lie at a central point in the multiple signal transduction pathways for various growth factors, hormones and cytokines. Extracellular signal-regulated protein kinase 1 and 2 (ERK1 and ERK2) requires phosphorylation of both Thr-183 and Tyr-185 for activation (23). We have previously shown that protein tyrosine kinase inhibitors suppress the LPS-induced expression of COX-2 and proinflammatory cytokines in macrophages and these PTK inhibitors also inhibit LPS-induced activation of MAPKs in the murine macrophage cell line RAW 264.7 (2). Parthenolide also suppressed LPS-stimulated tyrosine phosphorylation of various proteins in RAW 264.7 cells as assessed by antiphosphotyrosine immunoblot (Fig. 5A). Among these pro-

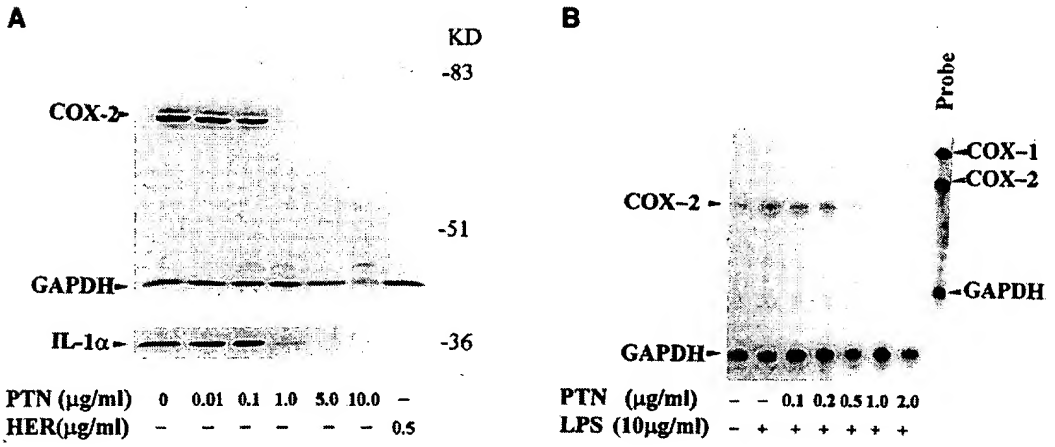

FIG. 3. (A) Dose-dependent inhibition of the expression of COX-2 and IL-1α proteins by parthenolide. Alveolar macrophages were incubated with LPS and various concentrations of parthenolide for 16h. Solubilized proteins were analyzed by COX-2, IL-1α or GAPDH immunoblotting. Representative immunoblot of more than five different analyses. PTN, parthenolide; HER, herbimycin A. (B) Dose-dependent inhibition of the steady state levels of COX-2 and GAPDH mRNAs by parthenolide. Alveolar macrophages were incubated with LPS in the presence of various concentrations of parthenolide for 2h. Steady state levels of mRNA were determined by RNase protection assay.

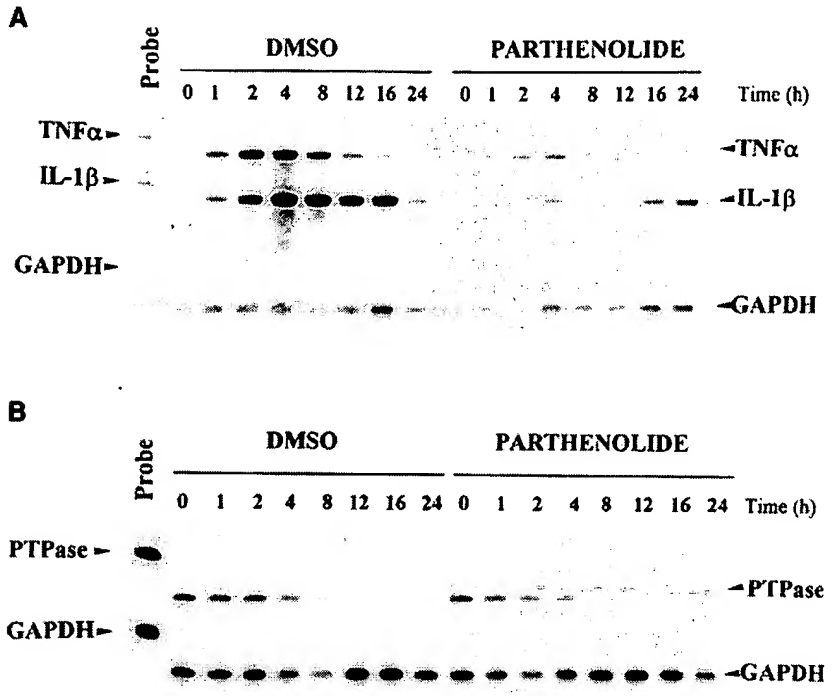

FIG. 4. Time course for steady state levels of mRNA for TNFα, IL-1β, PTPase and GAPDH. Alveolar macrophage were incubated with LPS in the presence of 1 μg/ml of parthenolide for specified time periods. Abundance of mRNA was determined by RNase protection assay.

teins MAPKs exhibited the most dramatic inhibition in the extent of tyrosine phosphorylation in response to parthenolide. The tyrosine phosphorylation of three MAPK subfamily (ERK1, ERK2 and P38) stimulated by LPS was inhibited by parthenolide in a dose dependent manner (Fig. 5A). The monoclonal antiphosphotyrosine antibody (4G10) does not recognize phosphorylated JNKs. Therefore, the extent of tyrosine phosphorylation of JNK-1 was assessed by electrophoretic mobility shift of phosphorylated JNK-1 as shown in Fig. 5B. Parthenolide inhibited tyrosine phosphorylation of JNK-1. Another protein tyrosine kinase inhibitor, herbimycin A, also inhibited tyrosine phosphorylation of MAPK subfamily. The inhibition of tyrosine phosphorylation of MAPKs correlated with the inhibition of COX-2 and IL-1α expression in RAW 264.7 cells (Fig. 5D). It has been demonstrated that the production of interleukin-1 and tumor necrosis factor from stimulated human monocytes is inhibited by the selective inhibitor of P38 (24). In our previous study, it was shown that the activation of MAPKs is required for the expression of COX-2 (25).

The mechanism by which parthenolide inhibits protein tyrosine phosphorylation is not known. It has been speculated that tyrosine kinase inhibitor, herbimycin A inactivates $p60^{v\text{-}src}$ kinase by irreversibly binding to the sulphydryl (SH) groups of $p60^{v\text{-}src}$ kinase (16). The inactivation may occur through conjugation between highly polarized double bonds in the benzoquinone moiety of herbimycin A and the SH group of sulphydryl compounds. Similarly, the α-methylenebutyrolactone in parthenolide can interact with biological nucleophiles such as sulphydryl groups. Indeed, pretreating cells with sulphydryl compounds abrogated the inhibitory effect of parthenolide on LPS-induced activation

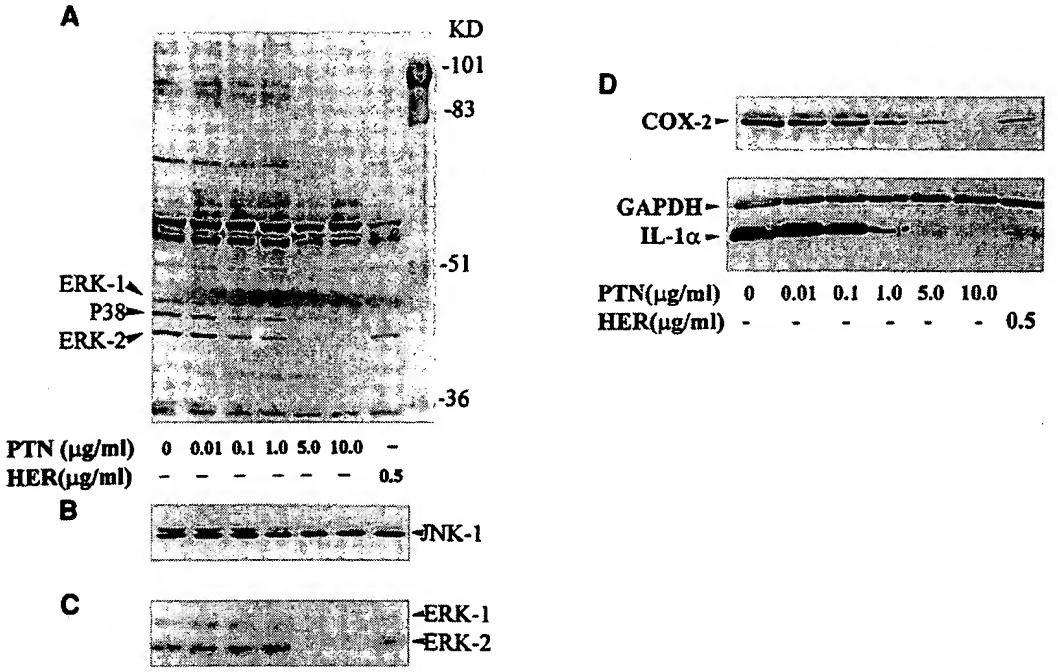

FIG. 5. Dose-dependent inhibition of protein tyrosine phosphorylation and kinase activity of ERK-1 and ERK-2 by parthenolide and herbimycin A. This inhibition correlates with the suppressed expression of COX-2 and IL-1α. RAW 264.7 cells were pretreated with parthenolide in various concentrations or herbimycin A (0.5 μg/ml) for 3h and then stimulated with LPS (1 μg/ml) in the presence of the inhibitors for 30 min. (A) Antiphosphotyrosine immunoblot. Representative immunoblot of more than five different analyses. (B) JNK-1 immunoblot of the same samples used in A. (C) In-gel kinase assay using myelin basic protein (MBP) as a substrate. (D) COX-2, IL-1α or GAPDH immunoblots. PTN, parthenolide; HER, herbimycin A.

of MAPKs (Fig. 6A). Furthermore, the abolishment of the inhibitory effect of parthenolide on the activation of MAPKs by these agents resulted in recovered expression of COX-2 and IL-1α that had been inhibited by parthenolide (Fig. 6B). These results imply that the inhibitory effects of parthenolide are mediated through conjugation with SH - groups of target proteins. However, these data alone do not permit us to identify specific target protein(s) that are affected by parthenolide. Whether parthenolide inhibits protein tyrosine phosphorylation by directly inhibiting PTKs or by inhibiting other target protein(s) that affects the activity of PTKs, is not known.

Structure-function relationship among various sesquiterpene lactones in inhibiting the expression of COX-2. Among sesquiterpenes tested, parthenolide, encelin and leucanthin B (1, 2, and 3, respectively in Table 1) showed the highest inhibitory activity. The common feature of the compounds with strong inhibitory activity is that they possess α-methylene-gamma-lactone moiety and/or epoxide. Compound 2 possesses three possible conjugation sites. Saturation of the 11, 13-double bond in parthenolide as seen in compounds 11, 12 and 13 resulted in loss of the inhibitory activity. These results suggest that the α-methylene-gamma-lactone moiety confers the inhibitory ability of these sesquiterpene lactones.

Conjugation can occur between highly polarized double bonds in the benzoquinone moiety of herbimycin A and SH groups of PTK (16). Similarly, conjugation of SH groups can occur at C-9 with a conjugated double bond or at C-5 and C-6 bearing an epoxide in

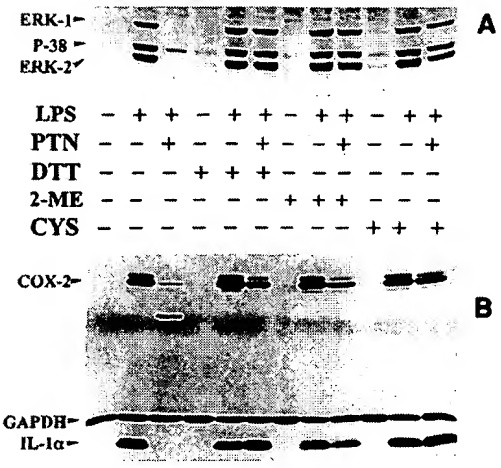

FIG. 6. Sulphydryl compounds abolish inhibitory effects of parthenolide on tyrosine phosphorylation of MAPKs and expression of COX-2 and IL-1α. RAW 264.7 cells were pretreated with parthenolide (1μg/ml) in the presence of DDT (dithiothreitol, 100μM), 2-ME (2-mercaptoethanol, 50μM) or Cys (L-cysteine, 150μM) and then stimulated with LPS (1μg/ml). Cells were incubated for 30 min for antiphosphotyrosine immunoblot analysis (A) and 8h for COX-2 and IL-1α Western blot analyses (B).

radicicol molecule (1). Therefore, it appears to be a general feature that many PTK inhibitors derived from natural products possess functional moieties that can confer conjugate addition reaction with biological nucleophiles such as SH group. Compounds 6, 8 and 9 lack the epoxide, and their inhibitory activity was diminished as compared with parthenolide. Whereas, compounds 3, 4 and 5 with additional epoxides in their molecules exhibited similar activity as parthenolide. However, compounds 11 and 12 containing an epoxide moiety without α-methylene-gamma-lactone moiety showed loss of the inhibitory activity. Therefore, the presence of epoxide moiety appears to accentuate the inhibitory

TABLE 1
Relative Potency of Different Sesquiterpene Lactones in Inhibiting the Expression of COX-2 in LPS-Stimulated Macrophage[a]

Compound No. in Fig. 1	Common name	Mol wt	(IC_{50} (μg/ml)
1	Parthenolide	248	0.2
2	Encelin	244	0.1
3	Leucanthin B	478	0.2
4	Enhydrin	464	0.3
5	Melampodin A	444	0.5
6	Confertiflorin	306	0.9
7	Burrodin	264	1.0
8	Psilostachyin A	280	1.3
9	Costunolide	232	1.6
10	Tenulin	306	5.5
11, 12, and 13			>100

[a] IC_{50} was determined at multiple dose levels.

activity of sesquiterpene lactones bearing α-methylene-gamma-lactone moiety, but epoxide moiety alone does not confer the inhibitory activity.

Endotoxic lipopolysaccharide (LPS) in gram negative bacteria is a primary component initiating septic shock. LPS activates macrophage, monocyte and neutrophil, and the activated cells produce proinflammatory cytokines and lipid mediators that mediate inflammatory responses. LPS stimulates protein tyrosine phosphorylation of MAPKs in macrophage. Suppression of the LPS-induced protein tyrosine phosphorylation by PTK inhibitors resulted in inhibition of the expression of proinflammatory cytokines and COX-2. Our finding that parthenolide inhibits tyrosine phosphorylation of MAPKs and the production of proinflammatory cytokines, offers a possibility that some of the sesquiterpene lactones can be used as a therapeutic agent for septic shock and other acute inflammatory diseases. Identifying the specific PTK inhibited by sesquiterpene lactone and elucidating the molecular mechanism by which these moieties exert the inhibitory effect will help designing specific PTK inhibitors with therapeutic potential.

ACKNOWLEDGMENTS

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REFERENCES

1. Chanmugam, P., Feng, L., Liou, S., Jang, B. C., Boudreau, M., Lee, J. H., Kwon, H. J., Beppu, T., Yoshida, M., Xia, Y., Wilson, C. B., and Hwang, D. (1995) *J. Biol. Chem.* **27,** 5418–5426.
2. Feng, L., Jang, B. C., and Hwang, D. (in press, 1996) *in* Eicosanoids and Other Bioactive Lipids in Cancer, Inflammation and Radiation Injury (Honn, K. V., Nigam, S., Jones, R., Marnette, L. J., and Wong, P. Y-K., Eds.), Plenum, NY.
3. Fantl, W. J., Johnson, D. E., and Williams, L. T. (1993) *Annu. Rev. Biochem.* **62,** 453–481.
4. Ullrich, A., and Schlessinger, J. (1990) *Cell* **61,** 203–212.
5. Rosen, O. M. (1987) *Science* **237,** 1452–1458.
6. Honegger, A. M., Szapary, D., Schmidt, R., Lyall, R., VonObberghen, E., Dull, T. T., Ullrich, A., and Schlessinger, J. (1987) *Mol. Cell Biol.* **7,** 4568–4571.
7. Yoneda, T., Lyall, R. M., Alsina, M. M., Persons, P. E., Spada, A. P., Levitzki, A., Zilberstein, A., and Mundy, G. R. (1991) *Cancer Research* **51,** 4430–4435.
8. Levitzki, A., and Gazit, A. (1995) *Science* **267,** 1782–1788.
9. Weinstein, S. L., Sanghera, J. S., Lemke, K., DeFranco, A. L., Pelech, S. L. (1992) *J. Biol. Chem.* **267,** 14955–14962.
10. Novogrodsky, A., Vanichkin, A., Patya, M., Gazit, A., Osherov, N., and Levitzki, A. (1994) *Science* **264,** 1319–1322.
11. Lee, S. H., Soyoola, E., Chanmugam, P., Hart, S., Sun, W., Zhong, H., Liou, S., Simmons, D., and Hwang, D. (1992) *J. Biol. Chem.* **267,** 25934–25938.
12. Graziani, Y., Chayoth, R., Karny, N., Feldman, B., and Levy, J. (1981) *Biochim. Biophys. Acta.* **714,** 415–421.
13. Akiyama, T., Ishida, J., Nakagawa, S., Ogawara, H., Watanabe, S. I., Itoh, N., Shibuya, M., and Yasuo, F., Genistein (1987) *J. Biol. Chem.* **262,** 5592–5595.
14. Onoda, T., Iinuma, H., Sasaki, Y., Hamada, M., Isshiki, K., Naganawa, H., Tekeuchi, T., Tatsuta, K., and Umezawa, K. (1989) *J. Nat. Prod.* **52,** 1252–1257.
15. Umezawa, H., Imoto, M., Sawa, T., Isshiki, K., Matsuda, N., Uchida, T., Iinuma, H., Hamada, M., and Takeuchi, T. (1996) *J. Antibiot (Toyko)* **39,** 170–173.
16. Uehara, Y., Fukazawa, H., Murakami, Y., and Mizuno, S. (1989) *Biochem. Biophys. Res. Commun.* **163,** 803–809.
17. Fry, D. W., Kraker, A. J., McMichael, A., Ambroso, L. A., Nelson, J. M., Leopold, W. R., Connors, R. W., and Bridges, A. J. (1994) *Science* **265,** 10093–1095.
18. El-Feraly, F. S., and Chan, Y. M. (1978) *J. Pharma. Sci.* **67,** 347–350.
19. Fischer, N. H., Olivier, E. J., and Fischer, H. D. (1979) *in* Prog. Chem. Org. Nat. Prod. (Herz, W., Grisebach, H., and Kirby, G. W., Eds.), Vol. 38, pp. 47–390, Springer, Wien.
20. Kameshita, I., and Fujisawa, H. (1989) *Anal. Biochem.* **183,** 139–143.
21. Charles, C. H., Abler, A. S., and Lau, L. F. (1992) *Oncogene* **7,** 187–190.

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22. Sun, H., Charles, C. H., Lau, L. F., and Tonks, N. K. (1993) *Cell* **75**, 487–493.
23. Payne, D. M., Rossomando, A. J., Martino, P., Erickson, A. K., Her, J. H., Shananowitz, J., Hunt, D. F., Weber, M. J., and Sturgill, T. W. (1991) *EMBO J.* **10**, 885–892.
24. Lee, J. C., Laydon, J. T., McDonnell, P. C., Gallagher, T. F., Kumar, S., Green, D., McNulty, D., Blumenthal, M. J., Heys, J. R., Landvatter, S. W., Strickler, J. E., McLaughlin, M. M., Siemens, I. R., Fisher, S. M., Livi, G. P., White, J. R., Adams, J. L., and Young, P. R. (1994) *Nature (London)* **372**, 739–746.
25. Hwang, D. H., Jang, B. C., Yu, G., Boudreau, M., Kim, J. K., and Wan Lee (unpublished data).
26. Aggarwal, B. B., Kohr, W. J., Hass, P. E., Moffat, B., Spencer, S. A., Henzel, W. J., Bringman, T. S., Nedwin, G. E., Goeddel, D. V., and Harkins, R. R. (1985) *J. Biol. Chem.* **260**, 2345–2354.

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